DATE: 09/27/2001 TIME: 12:11:57

Input Set : A:\Inteinm.app Output Set: N:\CRF3\09272001\I786009.raw 3 :110: APPLICANT: XU, Ming-Qun EVANS, Thomas C. r < 120). TITLE OF INVENTION: Intein Mediated Peptide Ligation 8 (130) FILE REFERENCE: NEB-150PUS 10 - 1400 CURRENT APPLICATION NUMBER: 09/786,009 C--> 11 <141> CURRENT FILING DATE: 2001-04-17 13 1500 PRIOR APPLICATION NUMBER: 60/102,413 ENTERED 14 | 151: PFIOR FILING DATE: 1999-09-30 16 (150) PRIOR APPLICATION NUMBER: PCT/US99/22776 17 11510 PRIOR FILING DATE: 1999-09-30 19 11600 NUMBER OF SEQ ID NOS: 9 21 -: 170: SOFTWARE: PatentIn Ver. 2.0 23 - (210) - SEQ ID NO: 1 24 -1211 LENGTH: 43 25 -12121- TYPE: DNA 26 (213) ORGANISM: Artificial Sequence 28 - 3200 FEATURE: . O BIR-OTHER INFORMATION: Description of Artificial Sequence: the modified Coterminal splice junction of the intein from the gyrA gene of Mydobacterium xenopi 31 33 (4)00 SEQUENCE: 1 43 34 ggttogtdag odaegdtact ggedtoadeg gttgataget gda 36 - 219 SEQ ID NO: 2 37 - 2111 - LENGTH: 39 38 (212) TYPE: DNA 39 Dl3: ORGANISM: Artificial Sequence DEG: FEATURE: 4.3 41 - 1223 CTHER INFORMATION: Description of Artificial Sequence: the 43 complementary strand of the C-terminal splice junction of the modified intein from the gyrA 💚 44 4.5 -gene of Mydobadterium xenopi 47 - 14 MGH SEQUENCE: 239 48 getateaach ggtgaggoba gtagegtgge tgaegaace 12175 SEQ ID NO: 3 04 ...11 - LENGTH: 68 1212 · TYPE: DNA . . . FGANISM: Artificial Sequence The Time FEATURE: .: OTHER INFORMATION: Description of Artificial Sequence: the polylinker sequence inserted upstream of the modified intellifrom the gyrA gene of Mycobacterium xenopi FIRST (400 - SEQUENCE: 3 +. Threat stag acotatoged atdogtoged geogeotogal gogotottod tocatology $60\,$ 6d gamatgca 64 (11) SEQ ID NO: 4 65 <211 · LENGTH: 69 55 KBIBS TYPE: DNA

RAW SEQUENCE LISTING

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69 -1220> FEATURE:
70 + 323 OTHER INFORMATION: At position 41, "H" = A or C or T.
72 - 3220 FEATURE:
73 (223) OTHER INFORMATION: Description of Artificial Sequence: the
74
        complementary strand of the polylinker inserted
75
        upstream of the modified intein from the gyrA
76
        gene of Mycobacterium xenopi
76 - 400: SEQUENCE: 4
7) studiteate tecography caggaagage ectegagged hydryceaec catggedata 60
80 tgtctagat
82 - 210 - SEQ ID NO: 5
83 - 2111 LENGTH: 6509
84 - 212 - TYPE: DNA
85 - 213 : ORGANISM: Artificial Sequence
87 - 2220> FEATURE:
88 - 1223: OTHER INFORMATION: Description of Artificial Sequence: pTXB1 plasmid
        sequence containing the modified intein from the
89
96
         gyrA gene of Mycobacterium xenopi
92 -(400) SEQUENCE: 5
9) auctaegica ggiggcacti ticggggaaa igigcgcgga acceptatit gittattiti 60
94 othaatabat toaaatatgt atdogotoat gagadaataa oootgataaa tgottoaata 120
So utatigaaaa aygaagadta tgaytattoa abatttoogt gtogoobtta ttoobttttt 180
96 tgoggoatti tgoottootg tittitgotoa oobagaaabg otggifgaaag taaaayatgo 240
97 tgaagatdag ttgggtgdad gagtgggtta datdgaadtg gatdtdaada goggtaagaf 300
98 octtqaqaqt tttcqccccq aagaacqttc tocaatqatq aqcactttta aagttctqct 360
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100 ctatteteag aatgaettgg ttgagtaete accagteaca gaaaageate ttaeggatgg 480
101 catqueagta agagaattat geagtgetge cataaccatg agtgataaca etgeggeeaa 540
102 chtacttotg acaacgatog gaggacogaa ggagotaaco gottttttgc acaacatggg 600
163 ggattatgta actogoottig atogttiggga acoggagotig aatgaagoda taccaaacga 660
154 equipped accase gatge of grage and greaterage transfer tatta actgg=720
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196 tgcaggacca ottotgcgct oggocottoc ggctggctgg tttattgctg ataaatotgg 840
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\pm 1.8 obgitatogia gittatotada ogadggggag idaggdaadi atggatgaab gaaatagaba 960.
1^{\circ}9 gatogotgag ataggtgoot cactgattaa geattggtaa otgtoagaec aagtttacte 1020
lio akatatatti tagattgatt taccccggtt gataatcaga aaagccccaa aaacaggaag 1080
1.1 attytataag paaatattta aattytaaab gftaatatti tyttaaaatt rypgttaaat 1140
111 tritigittaaa toagotoatt tiittaabbaa taggoogaaa toggoaaaat boottataaa 1200
1. A baaaaagaat agoobkaqat agoottgagt gttgttobag titggaabaa gagtobabta 1260
1.4 thawayaacy tygachocaa oqtoawaggy ogwawaaccy totahowyyy custodroca 1.26\,
   orungrapan dathammaa afdaagtitt tiggagtoga ggigoogtaa agdadtaaat 1380
116 bygaabbota aagygagobo bogalitaga qottjanggu 48834nnggb gaangiddog 1446
1.-44411394887 44447413996 mahaddaddd ggogotaggg ogotggoaag tyfagoggto 1.8\%
118 abgotgogog taabbadbad abbogbbgbg stiaktybji bjirkekku bybitaaaad 1560
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120 gttocactga gegtcagaco cegtagaaaa gatcaaayya lottottyiy atcettt### 1k80
121 totgogogia atotgotgot tgcaaacaaa aaaaccaccg ctaccagegg tggtttgttt 1740
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122 geoggateaa gagetaecaa etettittee gaaggtaaet gyetteagea gagegeagat 1800 123 accaaatact gteettetag tgtageegta gttaggeeac eactteaaga actetgtage 1860 124 acceptaca tacctogoto tectaateet ettaccaette ettectaeta ettectaeta 1920 125 gtogtgtott accegggttgg actoaagaog atagttacceg gataaggego agceggteggg 1980 126 otgaacgygg gyttogtyca cacagedday ettygagega acgaectaca eegaactyay 2040 127 atacetacag egtgagetat gagaaagege caegetteee gaagggagaa aggeggacag 2100 128 gtateeggta ageggeaggg teggaacagg agagegeaeg agggagette cagggggaaa 2160 129 egeetggtat etttatagte etgtegggtt tegecacete tgaettgage gtegattttt 2220 130 gtgatgeteg teagggggge ggageetatg gaaaaaegee ageaaegegg eetttttaeg 2280 131 gtteetggee tittgetgge citttgetea catgitetti eetgegitat eeeetgatie 2340 13. tyttggataac cytattaccy cotttgayty agetyatacc yetogeogea geogaacyae 2400 133 ogagogoago gaytoagtga gogaggaago tatggtgoad totoagtada atotgototg 2460 134 atgeografia gttaagocag tatacactee getategeta egtgaetggg teatggetge 2520135 geologicae cogeciaeae cogetgaege geologicaeg gettgtetge teleggeate 2580 136 ogottadaga daagotigtiga dogtotoogig gagotigdatig tigtoagaggit itticadogite 2640 137 atcaccgaaa cgcgcgaggc agctgcggta aagctcatca gcgtggtcgt gcagcgattc 2700 138 abagatgtot gootgttoat oogogtocag btogttgagt ttotocagaa gogttaatgt 2760 139 etggettetg ataaageggg ceatgttaag ggeggttttt teetgtttgg teaettgatg 2820 140 octoogtgta agggggaatt totgttoatg ggggtaatga taccgatgaa acgagagagg 2880 14) atgeteaega taegggttae tgatgatgaa eatgeeeggt taetggaaeg ttgtgagggt 2940 142 aaacaactgg cggtatggat goggogggab bagagaaaaa toactcaggg toaatgocag 3000 ± 1 k ongaalgoca golaagaogta goodagogog toggoogoda tgooggogat aatggootgo 3060144 ttotogosya aabgiittggi quoyggabba gigacgaagg bilgagiqag ggbgitgbaag 3120 145 attoogaata cogcaagoga caggoogato atogtogogo tocagogaaa giqqibootog 3080 145 cogaaaatga cocagagege tgooggcaco tgtoctacga gttgcatgat aaagaagaca 3040 147 gtdataagtg dggdgadgat agtdatgddd dgdgdddadd ggaaggagdt gadtgggttg 3300 148 aaggetetea agggeategg tegagateee ggtgeetaat gagtgageta aettaeatta 3360 149 attgegttge geteactgee egettteeag tegggaaace tgtegtgeea getgeattaa 3420 15% tgaateggee aaegegeggg gagaggeggt ttgegtattg ggegeeaggg tggtttttet 3480 151 titleaccagt gagaegggea acagetgatt gecetteace geotggeect gagagagttg 3540 152 dagdaagogg todacgotgg titigodocag daggogaaaa tootgiitiga iggiggitaa 3600 153 eggegggata taacatgage tgtetteggt ategtegtat eccaetaceg agatateege 3660 154 accasegoge ageologiset eggtastige gegestigeg eccagogoes teligategit 3720155 qqcaaccaqc atoqcaqtqq qaacqatqcc ctcattcaqc atttqcatqq tttqttqaaa 3780 156 acceggacate yeartopagt ogcottocce ttoccetato egetgaatit gattgegagt 3840 157 gagatattta tgobagooag obagaogoag aegogoogag abagaabtta atgggboogo 3900 155 taacaqoqoq atttqctqqt qacccaatgc qaccaqatgc tocacgccca gtcgcqtacc 3960 159 gtoftoatgg gagaagataa factgttgat gggtgtotgg toagagacat baagaaataa 4020 160 ogooggaada itagiigbagg bagbiibbbab agbaatggba ibbiiggibai bhagoggata 4080 16) gttaatgate ageccactga egegttgege gagaagattg tgeacegeeg etttabagge 4140 162 thogapypog ottogttota poatogabao naocaogotig goadboagtt gatoggogoy 4200 lfw agairtaato googogacaa trigogaogg ogogtgoagg gooagacigg agaiggoaac 40f6 2.4 godaatbago aargadigtt forhooddag togotgog aegogotgg gaatgtaatb 4%x. 1mb cagotoogoo atogoogott obabtttio objecttiin ypakkaaangt $\sigma\sigma$ nigdboid 4.78%int gtriackalg oggiskkrug forgahadda gadaboggod tabtotgogd batogtataa 444. Th/ ogttactggt ttdacattca ccaccotgaa ttgactcts: foogganist athermost 45% 168 accepagaaag gittigggrounitegafogf gicologgato tegacgetet ecottaigeg 4.6%169 aptentional taggaagpag penagtagta ggttgagged gttgage $x v \sim y c z g n c g n a 4 \kappa \omega \phi$ 170 ggaatggtgc atgeogeoot ttogteffea aqaattaatt edeaattoba ggoafeaaat 4680

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Input Set : A:\Inteinm.app

Output Set: N:\CRF3\09272001\I786009.raw

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173 eggagggtgg egggeaggae geoegecata aactgecagg aattaattee aggeateaaa 48 \mathrm{e}\, \mathrm{e}
174 taaaacgaaa ggctcagtcg aaagactggg cetttegttt tatetgttgt ttgtcggtga 4920
175 acgeteteet gagtaggaca aateegeegg gageggattt gaaegttgeg aagcaaegge 4980
176 coggagggtg gegggeagga egocegeeat aaactgeeag gaattaatte caggeateaa 5040
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179 cooggagggt ggogggoagg acgoocgoca taaactgoca ggaattaatt coaggoatoa 5220
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181 gaacqctctc ctgagtagga caaatcegec gggageggat ttgaacgttg cgaagcaacg 5340
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191 thacqcggtg attcaacgca gcgcattcag cgtcgactgt gcaggttttg cccgcgggaa 5940
1 \le 	anzerrange and the degree acade detaction and expression of the <math>1 \le 	anzerrange acts
Ira aqbababbab oqaqacooqq aqqbbbaaqqb tatbqbbqab qaqbbqabbq aqqqbqqtt 6060
194 chactacgog aaagtogoda gigicaccga ogobggogig cagooggigi atagootiing 612.
195 tytogacaeg geagaceaeg egittateae gaaegggite gicageeaeg etaetggeet 6.01
196 caccoquicty aacteagged teacgacaaa teetggtgta teegcittgge aggicaacac 6240
197 agottatact gogggacaat tggtcacata taacggcaag acgtataaat gtttgcagcc 6300
198 character tigghaggat gggaachath haangiteet goottgiggh agottbaatg 6360
199 actgraggaa ggggatoogg otgotaacaa agoocgaaag gaagotgagt tggotgotgo 6420
20) caccoctgag caataastag cataascott tggggsotet aaacgggtot tgaggggttt 6480
201 thtgotgaaa ggaggaacta tatccggat
203 <210> SEQ ID NO: 5
204 <0110 LENGTH: 30
2005 <2120 TYPE: PRT
20% KB13. ORGANISM: Artificial Sequence
208 <320 · FEATURE:
209 <223 - OTHER INFORMATION: Description of Artificial Sequence: synthetic
2.1
        peptide
212 - 400% SEQUENCE: 6
(41) Two Ala Tyr Lys Thr Thr Gln Ala Ash Lys His Ile Ile Val Ala Cys
                                         10
. . .
11: Gou Gly Ash Pro Tyr Val Pro Val His Fne Asp Ala Ser Val
                                     2.5
    .210. SEQ ID NO: 7
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III SANIA TYPE: PRT
135 (L13 - GRGANISM: Artificial Sequence
135 <320 → FEATURE:
Lue <223> OTHER INFORMATION: Description of Artificial Sequence: the amino acid
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Output Set: N:\CRF3\09272001\I786009.raw

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227
        sequence deduced from the polylinker region of
228
         pTXB1
236 <400> SEQUENCE: 7
231 Met Ala Met Gly Gly Gly Arg Leu Glu Gly Ser Ser Cys
232 1 5
235 + 210 \rightarrow SEQ ID NO: 8
236 211> LENGTH: 42
237 - 212> TYPE: DNA
238 - 213 > ORGANISM: Artificial Sequence
246 - 12200 FEATURE:
241 \pm 22.30 OTHER INFORMATION: Description of Artificial Sequence: polylinker -ar{\mathcal{U}}'
242
          region upstream of the modified intein from the
243
        gyrA gene of Mycobacterium xenopi in pTXB1
245 - 400 - SEQUENCE: 8
                                                                        42
246 catatggcca tgggtggcgg ccgcctcgag ggctcttcct gc
248 -22105 SEQ ID NO: 9
249 - 12111 - LENGTH: 7
250 -(212) TYPE: PRT
251 - 22130 ORGANISM: Artificial Sequence
253 - 220 FEATURE:
254 - 223 - OTHER INFORMATION: Description of Artificial Sequence: synthetic.
.355 peptide
257 HAGON SEQUENCE: 9
158 Cys Asp Pro Glu Lys Asp Ser
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259 1





VERIFICATION SUMMARY

PATENT APPLICATION: US/09/786,009 TIME: 12:11:58

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Input Set : A:\Inteinm.app

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L:11 M:271 C: Current Filing Date differs, Replaced Current Filing Date